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Mitochondrial impairment, apoptosis and autophagy in a rat brain as immediate and long-term effects of perinatal phencyclidine treatment — influence of restraint stress

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ABSTRACT

Phencyclidine (PCP) acts as a non-competitive antagonist of glutamatergic N-methyl-D-aspartate receptor. Its perinatal administration to rats causes pathophysiological changes that mimick some pathological features of schizophrenia (SCH). Numerous data indicate that abnormalities in mitochondrial structure and function could be associated with the development of SCH. Mitochondrial dysfunction could result in the activation of apoptosis and/or autophagy. The aim of this study was to assess immediate and long-term effects of perinatal PCP administration and acute restraint stress on the activity of respiratory chain enzymes, expression of apoptosis and autophagy markers and ultrastructural changes in the cortex and hippocampus of the rat brain.

Six groups of rats were subcutaneously treated on 2nd, 6th, 9th and 12th postnatal days (P), with either PCP (10 mg/kg) or saline (0.9% NaCl). One NaCl and one PCP group were sacrificed on P13, while other two NaCl and PCP groups were sacrificed on P70. The remaining two NaCl and PCP groups were subjected to 1 h restraint stress prior sacrifice on P70. Activities of respiratory chain enzymes were assessed spectrophotometrically. Expression of caspase 3 and AIF as markers of apoptosis and Beclin 1, p62 and LC3, as autophagy markers, was assessed by Western blot. Morphological changes of cortical and hippocampal ultrastructure were determined by transmission electron microscopy.

Immediate effects of perinatal PCP administration at P13 were increased activities of complex I in the hippocampus and cytochrome c oxidase (COX) in the cortex and hippocampus implying mitochondrial dysfunction. These changes were followed by increased expression of apoptotic markers. However the measurement of autophagy markers at this time point has revealed decrease of this process in cortex and the absence of changes in hippocampus. At P70 the activity of complex I was unchanged while COX activity was significantly decreased in cortex and increased in the hippocampus. Expressions of apoptotic markers were still significantly higher in PCP perinatally treated rats in all investigated structures, but the changes of autophagy markers have indicated increased level of autophagy also in both structures. Restraint stress on P70 has caused increase of COX activity both in NaCl and PCP perinatally treated rats, but this increase was lower in PCP group. Also, restraint stress resulted in decrease of apoptotic and increase of autophagy processes especially in the hippocampus of PCP perinatally treated group. The presence of apoptosis and autophagy in the brain was confirmed by transmission electron microscopy.

In this study we have demonstrated for the first time the presence of autophagy in PCP model of SCH. Also, we have shown increased sensitivity of PCP perinatally treated rats to restraint stress, manifested in alterations of apoptotic and autophagy markers. The future studies are necessary to elucidate the role of mitochondria in the pathophysiology of SCH and putative significance for development of novel therapeutic strategies.

Abbreviations: AIF, apoptosis-inducing factor; ANOVA, The Analysis Of Variance; COX, cytochrome c oxidase; DCIP, 2,6-dichloroindophenol; i.p., intraperitoneal; NMDA, N-methyl-D-aspartate; LC3, microtubule associated protein 1 light chain 3; P, postnatal day; PCP, phencyclidine; PV+, parvalbumin positive cells; SCH, schizophrenia; S.E.M., standard error of the mean; TEM, transmission electron microscopy.

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1. Introduction

Phencyclidine (PCP) is a noncompetitive antagonist of glutamatergic N-methyl-D-aspartate receptor (NMDA), with binding site in a receptor channel (Tsai and Coyle, 2002). Due to remarkable similarities between the experiences and symptoms of PCP abusers and patients suffering from schizophrenia (SCH), PCP has attracted a great attention of neuroscientists. Changes in glutamatergic system and NMDA receptor hypofunction have been proposed as an underlying cause of the pathophysiology of SCH (Javitt and Zukin, 1991; Olney et al., 1995; Olney and Faber, 1995; Coyle, 1996). There is also evidence that, in at least some cases, SCH may be a neurodevelopmental disorder involving a prenatal insult and/or genetic predisposition with delayed expression of clinical signs around the time of adolescence (Marenco and Weinberger, 2000). Perinatal PCP administration to rat pups induces neurodegeneration and long lasting behavioral disruption and represents an animal model of processes which may be involved in SCH (Radonjić et al., 2008; Wang et al., 2001). Biochemical, genetic and neuroimaging studies in humans suggest the involvement of mitochondrial dysfunction (Rajasekaran et al., 2015; Rezin et al., 2009) and apoptosis (Jarskog et al., 2005) in the pathophysiology of SCH. Also, recent studies have associated autophagy with SCH (Merenlender-Wagner et al., 2015), but precise mechanism has not been elucidated. It has been speculated that NMDA receptor excitotoxicity can act as a stressor to induce autophagy in neurons (Sadasivan et al., 2010).

The main energy-generating pathway in the brain is oxidative phosphorylation on the mitochondrial respiratory chain. The respiratory chain encompasses five multisubunit enzyme complexes named Complex I–V. Complex I remove electrons from NADH and transports it to coenzyme Q. Complex IV, named cytochrome c oxidase (COX), catalyzes the transfer of electrons to molecular oxygen (Wallace, 1994). Altered activity of complexes I and IV were identified *postmortem* (Maurer et al., 2001; Prabakaran et al., 2004) and in peripheral blood samples of SCH patients (Dror et al., 2002), as well as in ketamine animal model of SCH (Faizi et al., 2014; de Oliveira et al., 2011).

Mitochondrial dysfunction causes disturbed membrane permeability, dissipation of inner membrane potential, osmotic swelling of the matrix, rupture of outer mitochondrial membrane and cytochrome c releasing. Released cytochrome c activates the caspase 3 that is essential for apoptosis. *Postmortem* (Jarskog et al., 2004) and studies based on postnatal ketamine or PCP administration to rats (Scallet et al., 2004; Wang and Johnson, 2007; Anastasio et al., 2009) revealed alterations in caspase 3 level in the brain. On the basis of findings that administration of NMDA receptor antagonists to postnatal rodents induces neuronal death in specific brain regions, apoptosis has been suggested as neurodevelopmental insult involved in the pathophysiology of SCH (Ikonomidou et al., 1999; Jevtovic-Todorovic et al., 2003; Wang et al., 2001; Wang and Johnson, 2005). Apoptotic pathway could, also, be caspase-independent, triggered by apoptosis-inducing factor (AIF). Available evidence strongly suggests that AIF plays a crucial role in cell death in certain cell types, including neurons (Norberg et al., 2008; Wang et al., 2004; Cheung et al., 2005). In neurons AIF deficiency leads to oxidative damage and electron transport chain dysfunction probably indirectly affecting the complex I assembly or stability (Vahsen et al., 2004; Sevrioukova, 2011).

Autophagy blockade leads to cell death (Hara et al., 2006; Mortensen et al., 2010). Key proteins involved in autophagy pathway are Beclin 1, p62 and microtubule associated protein 1 light chain 3 (LC3) (Merenlender-Wagner et al., 2015). Beclin 1 is involved in autophagosomes formation by membrane recruitment (Cao and Klionsky, 2007) and it is suggested to be an important convergence point of autophagy and apoptosis (Merenlender-Wagner et al., 2015). The p62 is a protein that binds directly to the autophagy effectors LC3-I and LC3-II and its expression is negatively correlated with autophagy process (Pankiv et al., 2007; Kabeya et al., 2000). So far, autophagy has been investigated in neurodegenerative disorders (reviewed in Menzies et al., 2015) but the recent study has indicated the impairment of autophagy in the lymphocytes of SCH patients (Merenlender-Wagner et al., 2015). Investigations on animal models of SCH induced by administration of NMDA receptor antagonist are lacking.

Increased vulnerability to psychiatric disorders, including SCH, has been associated with higher levels of stress (Finlay and Zigmond, 1997). Animal studies indicate that chronic stress alters neuronal morphology in the prefrontal cortex (Wellman, 2001) and hippocampus (Magariños and McEwen, 1995). Maynard and colleagues have suggested the "two hits" hypothesis of SCH (Maynard et al., 2001) that states a "first hit", such as genetic predisposition or prenatal insult (e.g. exposure to NMDA receptor antagonist), disrupt brain development producing a long-term vulnerability to a "second hit" (e.g. social isolation, postnatal inflammation or drug abuse), that occurs later in life and is necessary for the full clinical syndrome onset (Bayer et al., 1999).

A precise relationship between perinatal NMDA receptor blockade and mitochondrial impairment, apoptosis and autophagy is not entirely understood. The aim of this study was to assess the immediate and long-term effects of perinatal PCP administration on the activity of cytochrome c oxidase and complex I, the expression of caspase 3 and AIF as markers of apoptosis, and the expression of autophagy markers Beclin 1, p62 and LC3 in the cortex and hippocampus of the rat brain. Morphological changes of the cortical and hippocampal ultrastructure were determined by transmission electron microscopy. Also, we have examined the influence of acute restraint stress on the same parameters in the brain of PCP perinatally treated rats.

2. Methods

2.1. Animals

Timed-pregnant Wistar rats were obtained at day 14 of pregnancy. The animals were housed individually in wire-hanging cages located within a temperature-controlled animal vivarium maintained under 12:12-h light/dark schedule (lights on at 07:00 h). Food and water were available ad libitum throughout the experiment. Within 12 h of parturition, the pups from dams were combined and then randomly assigned to one of the lactating dams. Day of birth was considered to be postnatal day (P) 0. Fifty animals were used in this study. Twenty five animals were treated on postnatal days (P) 2, 6, 9, and 12 with either phencyclidine (10 mg/kg) and the other twenty five with saline (NaCl 0.9%). Phencyclidine hydrochloride (Sigma, St. Louis, MO) was dissolved in a vehicle solution of 0.9% physiological saline (0.001 g/ml) and injected subcutaneously (s.c.) in the interscapular region. The dose and time course of the treatment were adapted from previously published studies (Adams et al., 2004; Ikonomidou et al., 1999; Wang et al., 2001). The vehicle control was saline alone injected s.c. in the same volume as PCP. Ten NaCl and ten PCP perinatally treated animals were sacrificed on P13, while the other ten NaCl and ten PCP perinatally treated rats were sacrificed on P70 (early adulthood) (Sengupta, 2013). Remaining five NaCl and five PCP perinatally treated animals were subjected to acute immobilization stress (restraint stress) on P70. Rats were completely immobilized for 1 h prior sacrifice in specially designed plastic restraint tubes (dimensions: 20 cm high, 7 cm diameter). All animals were sacrificed by cervical dislocation and decapitation without anesthesia. Heads were quickly frozen on liquid nitrogen and stored at −80 °C.

All efforts were made in order to minimize animal suffering and reduce the number of animals used in the study. All experiments were carried out according to the NIH Guide for Care and Use of Laboratory Animals and were approved by the Local Bioethics Committee.

2.2. Brain preparation for measurement of respiratory chain enzyme activity

For the determination of enzyme activity dorsolateral frontal cortex and hippocampus were dissected and the crude synaptosomal and